CLAIMS

1. A high throughput assay for detecting GST alleles present in a patient comprising the steps of:

obtaining a biological sample from the patient;

isolating genomic DNA from the sample;

performing PCR amplification of a portion of the DNA to detect GSTM1 alleles;

performing PCR amplification of a portion of the DNA to detect GSTM3 and GSTT1 alleles;

performing PCR amplification of a portion of the DNA to detect GSTP1 polymorphisms; and

detecting GSTM1, GSTM3, GSTT1, and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps.

2. The method of claim 1, wherein the biological sample is obtained from peripheral blood.

3. The method of claim 1, wherein the biological sample is obtained from blood spotted onto filter paper.

4. The method of claim 3, wherein the biological sample is obtained from a Guthrie card.

5. The method of claim 1, wherein the biological sample is obtained from buccal epithelial cells.

6. The method of claim 5, wherein the buccal epithelial cells are obtained from expectorated mouthwash.

7. The method of claim 5, wherein the buccal epithelial cells are obtained from buccal swabs.

8. The method of claim 1, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTM1 alleles comprises performing fluorescent, allele-specific PCR using GSTM1-specific primer sequences.

9. The method of claim 8, wherein individual GSTM1-specific primer sequences separately include the sequences of SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 5.

10. The method of claim 9, wherein the individual GSTM1-specific primer sequences are SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4.

11. The method of claim 8, wherein the portion of the DNA is also PCR-amplified to detect ß-actin as a reaction control using ß-actin-specific PCR primer sequences.

12. The method of claim 11, wherein the individual ß-actin-specific primer sequences separately include SEQ ID NO: 6 and SEQ ID NO: 8.

13. The method of claim 12, wherein the individual ß-actin-specific primer sequences are SEQ ID NO: 6 and SEQ ID NO: 7.

14. The method of claim 1, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTM3 and GSTT1 alleles comprises performing PCR using GSTM3- and GSTT1-specific primer sequences.

15. The method of claim 14, wherein the individual GSTM3- and GSTT1-specific primer sequences separately include SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 24 and SEQ ID NO: 26.

16. The method of claim 15, wherein the individual GSTM3- and GSTT1-specific primer sequences are SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 24 and SEQ ID NO: 25.

17. The method of claim 1, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTP1 polymorphisms comprises performing fluorescent, allele-specific PCR using GSTP1-specific primer sequences.

18. The method of claim 17, wherein the individual GSTP1-specific primer sequences separately include SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22 and SEQ ID NO: 23.

19. The method of claim 18, wherein the individual GSTP1-specific primer sequences are SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21 and SEQ ID NO: 23.

20. The method of claim 1, wherein the step of detecting GSTM1, GSTM3, GSTT1, and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps includes combining the DNA obtained from the PCR amplification steps to detect GSTM1, GSTM3, GSTT1, and GSTP1 alleles.

21. The method of claim 20, wherein the step of detecting GSTM1, GSTM3, GSTT1 and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps further includes conducting a gel electrophoresis of the combined DNA.

22. The method of claim 21, wherein GSTM1, GSTM3, GSTT1 and GSTP1 alleles are detected using PCR product size differences and fluorescent tag differences.

23. The method of claim 20, wherein the step of detecting GSTM1, GSTM3, GSTT1 and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps further includes conducting a capillary electrophoresis of the combined DNA.

24. The method of claim 23, wherein GSTM1, GSTM3, GSTT1 and GSTP1 alleles are detected using PCR product size differences and fluorescent tag differences.

25. The method of claim 24, wherein the step of detecting GSTM1, GSTM3, GSTT1, and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps is followed by performing a long range PCR assay of a portion of the DNA to distinguish GSTM1*A/A or GSTM1*B/B homozygotes from GSTM1*A/null and GSTM1*B/null heterozygotes.

26. The method of claim 25, wherein the step of performing a long range PCR assay of a portion of the DNA is conducted using GSTM1*0-specific primer sequences.

27. The method of claim 26, wherein the GSTM1*0-specific primer sequences are SEQ ID NO: 27 and SEQ ID NO: 28.

28. The method of claim 24, wherein the step of detecting GSTM1, GSTM3, GSTT1 and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps is followed by performing a long range PCR assay of a portion of the DNA to determine the gene dosage of GSTT1.

29. The method of claim 28, wherein the step of performing a long range PCR assay of a portion of the DNA to determine the gene dosage of GSTT1 is conducted using GSTT1*0-specific primer sequences.

30. The method of claim 29, wherein the GSTT1*0-specific primer sequences are SEQ ID NO: 33 and SEQ ID NO: 34.

31. The method of claim 28, wherein the step of performing a long range PCR assay of a portion of the DNA to determine the gene dosage of GSTT1 is conducted using GSTT1*0-specific primer sequences and GSTT1/GSTT2-non-specific primer sequences.

32. The method of claim 31, wherein the GSTT*0-specific primer sequences are SEQ ID NO: 33 and SEQ ID NO: 34 and the GSTT1/GSTT2-non-specific primer sequences are SEQ ID NO: 31 and SEQ ID NO: 32.

33. The method of claim 1, wherein the steps of performing PCR amplification of a portion of the DNA are followed by the steps of identifying portions of the DNA which failed PCR amplification and performing single nucleotide extension verification assays on the portions of the DNA which failed PCR amplification.

34. The method of claim 1, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTM1 alleles includes using primers having the sequences of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4; the step of performing PCR amplification of a portion of the DNA to detect GSTM3 and GSTT1 alleles includes using primers having the sequences of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 24 and SEQ ID NO: 25; and performing PCR amplification of a portion of the DNA to detect GSTP1 polymorphisms includes using primers having the sequences of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21 and SEQ ID NO: 23.

35. The method of claim 34 wherein the steps of performing PCR amplification of a portion of the DNA are followed by the steps of identifying portions of the DNA which

failed PCR amplification and performing single nucleotide extension verification assays on the portions of the DNA which failed PCR amplification.

36. A method of assessing the potential toxicity of chemotherapy in a patient comprising the steps of:

obtaining a biological sample from the patient;

isolating genomic DNA from the sample;

performing PCR amplification of a portion of the DNA to detect GSTM1 alleles;

performing PCR amplification of a portion of the DNA to detect GSTM3 and GSTT1 alleles;

performing PCR amplification of a portion of the DNA to detect GSTP1 polymorphisms;

detecting GSTM1, GSTM3, GSTT1, and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps; and

comparing the GSTM1, GSTM3, GSTT1 and GSTP1 polymorphic alleles present to predetermined standards to evaluate the potential toxicity of chemotherapy to the patient.

37. The method of claim 36, wherein the biological sample is obtained from peripheral blood.

38. The method of claim 36, wherein the biological sample is obtained from blood spotted onto filter paper.

39. The method of claim 38, wherein the biological sample is obtained from a Guthrie card.

40. The method of claim 36, wherein the biological sample is obtained from buccal epithelial cells.

41. The method of claim 40, wherein the buccal epithelial cells are obtained from expectorated mouthwash.

42. The method of claim 40, wherein the buccal epithelial cells are obtained from buccal swabs.

43. The method of claim 36, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTM1 alleles comprises performing fluorescent, allele-specific PCR using GSTM1-specific primer sequences.

44. The method of claim 43, wherein individual GSTM1-specific primer sequences separately include the sequences of SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 5.

45. The method of claim 44, wherein GSTM1-specific primer sequences are SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4.

46. The method of claim 43, wherein the portion of the DNA is also PCR-amplified to detect ß-actin as a reaction control using ß-actin-specific PCR primer sequences.

47. The method of claim 46, wherein the individual ß-actin-specific primer sequences separately include SEQ ID NO: 6 and SEQ ID NO: 8.

48. The method of claim 47, wherein the individual ß-actin-specific primer sequences are SEQ ID NO: 6 and SEQ ID NO: 7.

49. The method of claim 36, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTM3 and GSTT1 alleles comprises performing PCR using GSTM3- and GSTT1-specific primer sequences.

50. The method of claim 49, wherein the individual GSTM3- and GSTT1-specific primer sequences separately include SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 24 and SEQ ID NO: 26.

51. The method of claim 50, wherein the GSTM3- and GSTT1-specific primer sequences are SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 24 and SEQ ID NO: 25.

52. The method of claim 36, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTP1 polymorphisms comprises performing fluorescent, allele-specific PCR using GSTP1-specific primer sequences.

53. The method of claim 52, wherein the individual GSTP1-specific primer sequences separately include SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22 and SEQ ID NO: 23.

54. The method of claim 53, wherein the GSTP1-specific primer sequences are SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21 and SEQ ID NO: 23.

55. The method of claim 36, wherein the step of detecting GSTM1, GSTM3, GSTT1, and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps includes combining the portions of the DNA amplified to detect GSTM1, GSTM3, GSTT1, and GSTP1 alleles.

56. The method of claim 55, wherein the step of detecting GSTM1, GSTM3, GSTT1 and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps further includes conducting a gel electrophoresis of the combined DNA.

57. The method of claim 56, wherein GSTM1, GSTM3, GSTT1 and GSTP1 alleles are detected using PCR product size differences and fluorescent tag differences.

58. The method of claim 55, wherein the step of detecting GSTM1, GSTM3, GSTT1 and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps further includes conducting a capillary electrophoresis of the combined DNA.

59. The method of claim 58, wherein GSTM1, GSTM3, GSTT1 and GSTP1 alleles are detected using PCR product size differences and fluorescent tag differences.

60. The method of claim 59, wherein the step of detecting GSTM1, GSTM3, GSTT1, and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification steps is followed by performing a long range PCR assay of a portion of the DNA to distinguish GSTM1*A/A or GSTM1*B/B homozygotes from GSTM1*A/null and GSTM1*B/null heterozygotes.

61. The method of claim 60, wherein the step of performing a long range PCR assay of a portion of the DNA is conducted using GSTM1*0-specific primer sequences.

62. The method of claim 61, wherein the GSTM1-specific primer sequences are SEQ ID NO: 27 and SEQ ID NO: 28.

63. The method of claim 60, wherein the step of detecting GSTM1, GSTM3, GSTT1 and GSTP1 polymorphic alleles in the DNA obtained from the PCR amplification

steps is followed by performing a long range PCR assay of a portion of the DNA to determine the gene dosage of GSTT1.

64. The method of claim 63, wherein the step of performing a long range PCR assay of a portion of the DNA to determine the gene dosage of GSTT1 is conducted using GSTT1*0-specific primer sequences.

65. The method of claim 64, wherein the GSTT1*0-specific primer sequences are SEQ ID NO: 33 and SEQ ID NO: 34.

66. The method of claim 63, wherein the step of performing a long range PCR assay of a portion of the DNA to determine the gene dosage of GSTT1 is conducted using GSTT1*0-specific primer sequences and GSTT1/GSTT2-non-specific primer sequences.

67. The method of claim 66, wherein the GSTT*0-specific primer sequences are SEQ ID NO: 33 and SEQ ID NO: 34 and the GSTT1/GSTT2-non-specific primer sequences are SEQ ID NO: 31 and SEQ ID NO: 32.

68. The method of claim 36, wherein the steps of performing PCR amplification of a portion of the DNA are followed by the steps of identifying portions of the DNA which failed PCR amplification and performing single nucleotide extension verification assays on the portions of the DNA which failed PCR amplification.

69. The method of claim 68, wherein the step of performing PCR amplification of a portion of the DNA to detect GSTM1 alleles includes using primers having the sequences of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4; the step of performing PCR amplification of a portion of the DNA to detect GSTM3 and GSTT1 alleles includes using primers having the sequences of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 24 and SEQ

ID NO: 25; and performing PCR amplification of a portion of the DNA to detect GSTP1 polymorphisms includes using primers having the sequences of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21 and SEQ ID NO: 23.

70. The method of claim 69, wherein the steps of performing PCR amplification of a portion of the DNA are followed by the steps of identifying portions of the DNA which failed PCR amplification and performing single nucleotide extension verification assays on the portions of the DNA which failed PCR amplification.